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(54) Title: HUMAN BRAIN ENDOTHELIAL CELLS AND GROWTH MEDIUM AND METHOD FOR EXPANSION OF PRIMITIVE CD34+CD38– BONE MARROW STEM CELLS

(57) Abstract

A novel co–culture system using human brain endothelial cells (HUBEC) which promotes the expansion of human $CD34^+CD38^-$ cells consistent with the PMVEC system is disclosed. HUBEC were isolated from cadaveric donors, passed in primary culture, cloned and found to be Von Willebrand Factor positive. Cultivation of purified bone marrow $CD34^+$ cells on HUBEC monolayers supplemented with GM–CSF+IL–3+IL–6+SCF+flt–3 ligand caused a 14.5–fold increase in total cells, an 6.6–fold increase in $CD34^+$ cells, and, most remarkably, a 440–fold increase in $CD34^+CD38^-$ cells after 7 days. Further, CFU–GM production increased 15.1–fold, BFU–E increased 8–fold and CFU–Mix increased 5.2–fold. Optimal generation was dependent upon the continued presence of exogenous supplied cytokines. In comparison, identically treated stroma–free suspension cultures supported a 10.2–fold expansion of total cells, a 3–fold increase in $CD34^+$ cells and maintained the $CD34^+CD38^-$ cell pool after 7 days of culture. Moreover, we found that non–brain human endothelial cells isolated from the same donors supported neither the expansion nor the maintenance of human $CD34^+CD38^-$ cells. Although few steady state $CD34^+CD38^-$ cells give rise to visible colony–forming cells in methylcellulose cultures, our FACS based cell cycle and sorting experiments demonstrated the activation of a highly clonogenic $CD34^+CD38^-$ population (24 % cloning efficiency) during ex–vivo culture on cytokine treated HUBEC. These results suggest that bone marrow $CD34^+CD38^-$ cells require a stromal cell microenvironment for optimal expansion and that ex–vivo expanded $CD34^+CD38^-$ cells generated in the HUBEC culture system appear to retain some degree of primitive "stemness".

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/08 A61K35/28 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. CHUTE ET AL.: "Human brain microvascular endothelial cells promote a potent ex vivo expansion of primitive human hematopoietic progenitor cells." BLOOD, vol. 92, no. 10 suppl. 1 (part 1 of 2), 15 November 1998 (1998-11-15), page 584a XP000929910 Philadelphia, VA, USA abstract # 2406 ---	1-20
X	WO 95 11692 A (THE UNITED STATES OF AMERICA) 4 May 1995 (1995-05-04) page 11, line 5 - line 10 examples claims ---	1-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	J. CHUTE ET AL.: "Ex vivo expanded human CD34+ progenitor cells retain their primitive cell adhesion molecule profile when co-cultured with porcine microvascular endothelial cells and exposed to cytokines." EXPERIMENTAL HEMATOLOGY, vol. 25, no. 8, August 1997 (1997-08), page 807 XP000929911 Lawrence abstract # 271 ---	1-20
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